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BIOLOGICAL ACTIVITY OF SOILS STRONGLY POLLUTED WITH SULPHUR

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The studies were carried out on soils strongly polluted with sulphur and acidified (to pH 1.4). The soils were subjected to an intensive liming. In field and pot experiments, the authors determined: the total quantity of bacteria, actinomycetes, fungi, azotobacter, nitrifiers, proteolytic activity of microorganisms, activity of ammonifiers and the number of sulphur-oxidizing and sulphate-reducing bacteria. It was found that intensive liming of sulphur-affected soils restored their biological activity.

The studies were carried out on soils destroyed by exploitation of sulphur with a drilling method using steam under pressure (by smelting). Sulphur-affected soils are found in mining fields and in the adjacent zones. The content of sulphur in the exploited mining fields varies within wide limits. The lowest sulphur content found in those areas is high enough to make life of higher plants impossible.

Plants are also destroyed in the zone adjacent to the mining fields, which is polluted with sulphur dust. The sulphur contained in the soils undergoes a quick oxidation decreasing pH reaction to 1.5. Such a soil becomes completely barren. As there are large areas of sulphur deposits, its exploitation involves therefore several thousand hectares of soil. There arose thus an urgent need of developing a method of restoring biological life in the soils polluted with sulphur in order to make them agriculturally productive. First of all neutralization of sulphuric acid by intensive liming is necessary. This treatment helps restore the soils to agriculture. The authors carried out microbiological examinations, the purpose of which was to determine the rate and degree of biological activation of sulphur-affected soils after their having been limed.

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METHODS

In the microbiological examinations, soils from some pot and field experiments were taken into consideration.

Pot experiments were carried out on soil strongly polluted with sulphur (loamy sand from horizon A_1) from the mining field in Grzybów; and, on slightly loamy, sandy soil (Osiny near Puławy, horizon A_1) which was not initially polluted, with successive additions of native sulphur in the following amounts: O; 0.1%; 0.5%. In all four variants of soils — three polluted with sulphur and one without sulphur additions — were examined. To the soils were added the following amounts of calcium carbonate for each pot (7 kg of soil): (a) loamy, sandy soil from a mining field — 280 and 350 g of $CaCO_3$; (b) unpolluted, slightly loamy soil artificially polluted with sulphur; 140 g of $CaCO_3$ (the scheme of experiments is given in Table 1).

The soil for pot experiments was prepared (limed) in December, 1968. In the spring of the following year rape was sown, and in autumn, wheat. From the beginning of the experiments until the collection of samples (3rd January, 1970), the

TABLE 1. REACTION, CONTENT OF SOME MINERAL COMPONENTS AND PLANT GROWTH IN POT AND FIELD EXPERIMENTS

Experiment No.	% S	$CaCO_3$ (g per pot) (q per ha)	pH in H_2O	Total N %	Available components mg in 100 g			Plant species and condition
					P_2O_5	K_2O	Mg	
Pot experiment								
(a) loamy sand from the mining field (horizon A_1)								
1	1.87	0	1.6	0.18	4	100	48	wheat, perished
2	1.41	280	2.9	0.17	44	85	51	wheat, perished
3	1.41	350	6.4	0.11	57	35	23	wheat, very good
(b) slightly loamy sand (horizon A_1) with sulphur addition								
4	0.0	0	7.2	0.06	69	35	14	wheat, good
5	0.1	0	3.4	0.09	62	92	21	wheat, perished
6	0.1	140	7.2	0.06	72	35	15	wheat, good
7	0.5	0	2.0	0.12	67	121	51	wheat, perished
8	0.5	140	6.6	0.09	63	42	25	wheat, very good
Field experiment								
(a) in the mining field loamy sand with underlying loam								
9	3.87	1500	1.4	0.05	3	8	8	wheat, perished
10	1.39	1500	7.1	0.07	7	7	20	wheat, very good
11	0.84	1500	7.3	0.07	9	8	25	wheat, very good
12	2.72	1500	4.0	0.07	5	14	44	wheat, perished
13	1.27	1500	7.1	0.07	5	9	20	rye, very good
(b) in the protective zone, loamy sand with underlying loam								
14	0.26	0	2.5	0.08	5	4	11	wheat, perished
15	0.38	300	6.9	0.08	6	4	4	wheat, very good
16	0.25	300	5.7	0.08	4	7	2	rape, very good

following amounts of several compounds were applied for each pot: N— 1500 mg; P_2O_5 — 850 mg; K_2O — 1150 mg.

Field experiments were carried out in exploited mining areas and in the protective zone (adjacent to the mining fields). The microbiological examinations were carried out: (a) in the mining field with unlimed soil, and on limed soil sown with wheat and rye; (b) in the protective zone: on unlimed soil and on limed soil sown with rape and wheat.

Soil samples for the examinations were taken from the top layer (1–3 cm) of soil on June 12, 1970.

In those soils the authors determined:

— the total amount of bacteria and actinomycetes on agar medium with a soil extract;

— the amount of fungi on Martin's agar medium [5];

— the number of azotobacter on agar plates with saccharose, according to Fenglerowa's method [3] modified by Maliszewska.

— the number and activity of proteolytic organisms and ammonifiers, nitrifiers, bacteria-oxidizing and reducing sulphur, according to Pochon's method [7].

The microbiological determinations were done in 3–5 replicas, with the use of several dilutions. The culture was kept at 28–30°C. The content of following mineral components was determined: N — after Kjeldahl; S — after Butters and Chenery [1]; P_2O_5 and K_2O after Egner-Riehm [4]; Mg after Schachtschabel [4].

RESULTS

Sulphur content, pH of soils, the content of nutrients, the amount of the lime used and plant conditions in the experiments are presented in Table 1. These data show a distinct relationship between soil reaction and plant development. All plants were completely destroyed on the soil in which pH was 4 or lower.

The total number of bacteria and actinomycetes. In strongly acid soils ($pH \leqslant 4$) only a small number or a complete lack of bacteria and actinomycetes was found (Fig. 1). These organisms did not appear at $pH \leqslant 3.4$, whereas at pH 4 about one thousand of them were found. Particular attention was attracted by pot experiment No. 3, in which a small number of bacteria and actinomycetes were found, although pH of the soil was 6.4 and the condition of wheat was very good. Hence it appears that the soil sterilized before was not inhabited by microorganisms yet.

Fungi appeared both in pot and field experiments. At $pH \leqslant 2.5$, the number of fungi did not exceed 100 cells per lg of soil (Figs. 2 and 3). The greatest number of fungi (65,000/1 g of soil) was found in field experiment No. 12, where soil pH (in KCl) was 4 and wheat completely perished. In pot experiments, fungi grew best on the soil from a mining field neutralized to pH 6.4.

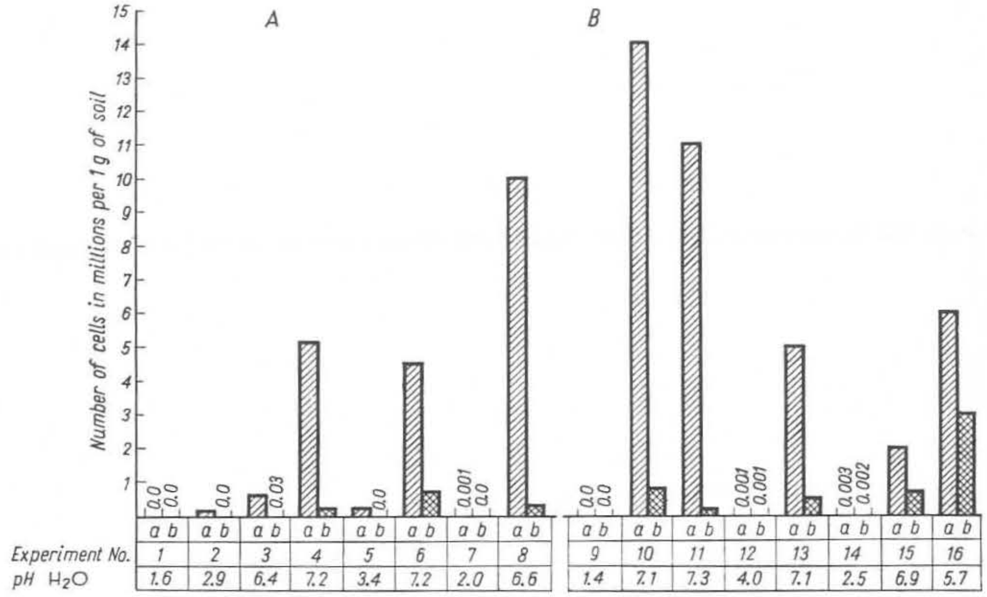

Fig. 1. Total number of bacteria and actinomycetes in soils of pot (*A*) and field (*B*) experiments. *a* — bacteria, *b* — actinomycetes; *1*, *2*, *3*, ... *16* — experiment No. (see Table 1).

In highly acidic soils (pH ⩽ 4), there appeared almost exclusively fungi of the genus *Penicillium*. In other field experiments and in pot experiment No. 3 (on neutralized soil from the mining field) the authors also found fungi of the genera: *Fusarium*, *Cladosporium*, *Macrosporium* and *Trichoderma*. Besides *Penicillium* there appeared *Aspergillus* on slightly loamy, sandy soil, with sulphur added without lime (pot experiment No. 5).

Azotobacter was found only in slightly loamy sand soil with the addition of 0.1% S and 2% $CaCO_3$ (140 g for each pot). Its absence in other soils is explained by their acid reaction before liming.

Proteolytic activity of microorganisms was the highest in neutralized soils polluted with sulphur.

An exception was slightly loamy, sandy soil with the addition of 0.1% S and 2% $CaCO_3$, the proteolytic activity of which was lower than that of the same soil in its initial stage.

A certain exception, however, was a very weakly deacidified soil from the mining field (pH — 2.9), in which proteolytic activity was relatively high (Fig. 4). Fungi were assumed to be active here (Fig. 2 and 4). A complete lack of proteolytic activity was found in soils strongly polluted with sulphur at pH ⩽ 2.0 (Table 1, Fig. 4).

The activity of ammonifiers was relatively high in all soils at pH > 2.5 and it increased in relation to decreasing acidity. Soils at pH 5.9 already showed a very high activity of ammonifiers (Fig. 5, Table 1).

Nitrifiers (nitrosobacteria and nitrobacteria) were present only in soils

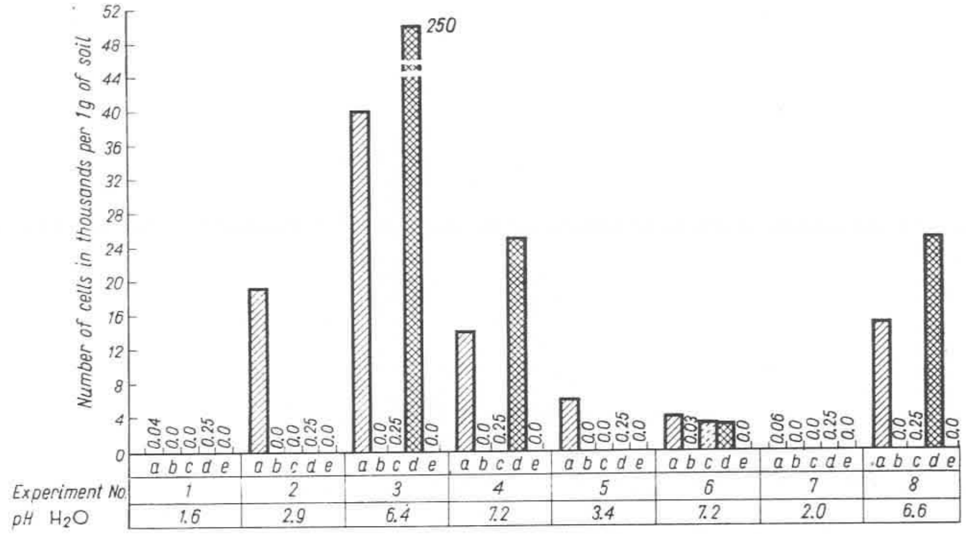

Fig. 2. Number of microorganisms in soil of pot experiments, *a* — fungi, *b* — azotobacter, *c* — nitrifiers, *d* — sulphur-oxidizing bacteria, *e* — sulphate-reducing bacteria; *1, 2, 3, ... 8* — experiment No. (see Table 1).

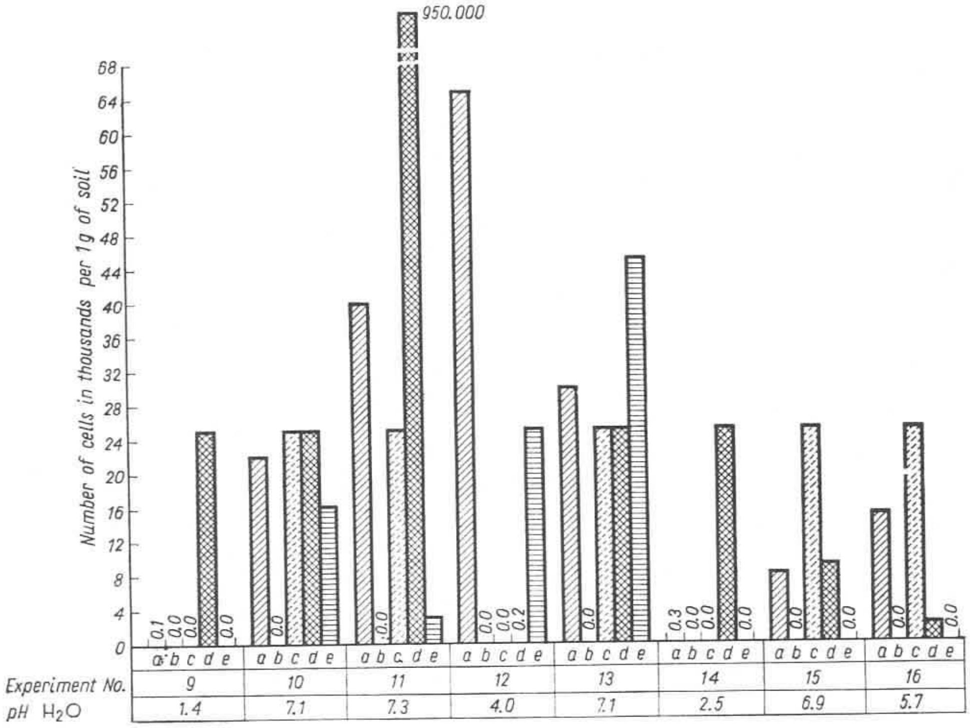

Fig. 3. Number of microorganisms in soils of field experiments. *a* — fungi, *b* — azotobacter, *c* — nitrifiers, *d* — sulphur-oxidizing bacteria, *e* — sulphate-reducing bacteria; *9, 10, 11, ... 16* — experiment No. (see Table 1).

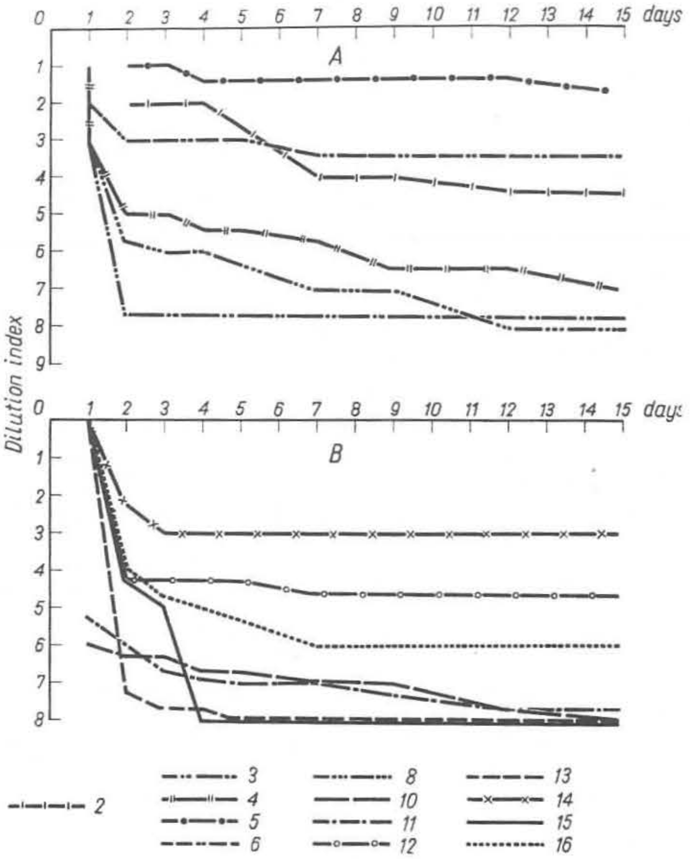

Fig. 4. Proteolytic activity of microorganisms in soils of pot (*A*) and field (*B*) experiments. *2*, *3*, *4*, ... *16* — experiment No. (see Table 1).

at pH ⩾ 5.7, i.e. where cultivated plants grew normally. No relationship between soil reaction and the number of nitrifiers at soil pH ⩾ 5.7 was found. An exceptionally great number of nitrifiers was found, with the addition of 0.1% S and 2% $CaCO_3$ (pH — 7.2, experiment No. 6), in sandy soil.

The presence of sulphur-oxidizing bacteria was found in all soils, but their number ranged within broad limits. Soils strongly acidified contained small numbers of these bacteria. An exception was soil strongly polluted with sulphur (pH — 1.4; exp. No. 9) in the mining field, in which (per 1 g of soil) about 25,000 sulphur-oxidizing bacteria were found. This, however, is not much in comparison with the same soil, limed, which contained about 0.25 million in a pot experiment (pH — 7.3), and about 9.5 milliard per 1 g of soil in field experiment No. 11 (pH — 7.3).

Sulphate-reducing bacteria did not appear in soils of the pot experiments except in weakly neutralized soil from the mining field (experiment 2).

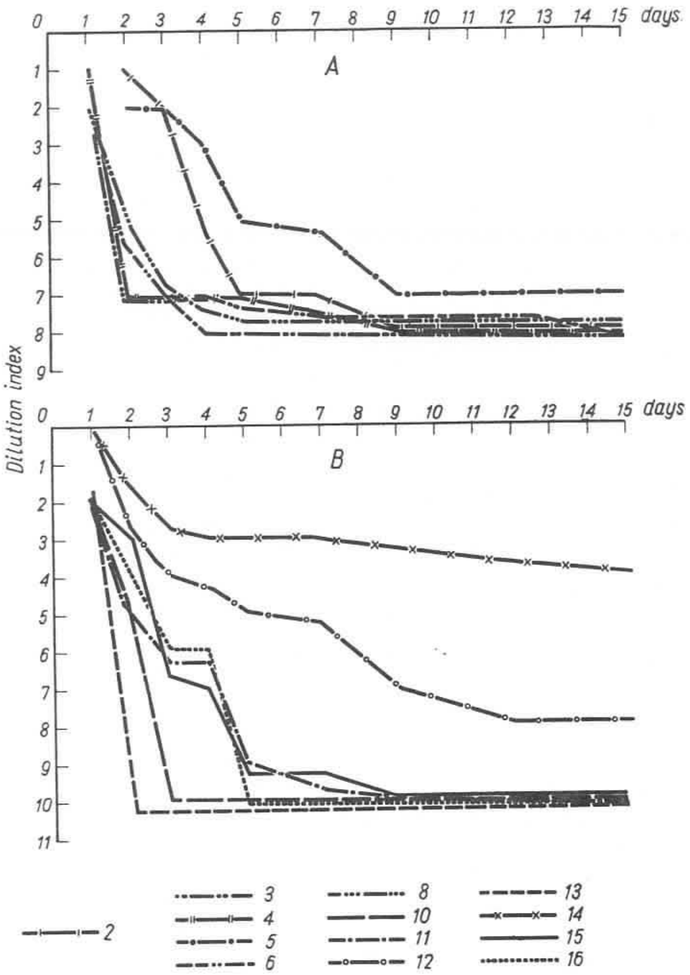

Fig. 5. Activity of ammonifiers in soils of pot (*A*) and field (*B*) experiments. *2*, *3*, *4*, ... *16* — experiment No. (see Table 1).

In the experiments (in the mining field) a considerable number of sulphate-reducing bacteria was found. A very small number of sulphate-reducing bacteria, however, was found in unlimed soil from the mining field (experiment No. 9).

DISCUSSION

The soil conditions dealt with are rarely encountered, and therefore literature with a comprehensive discussion of them is unavailable. Much is known about oxidation of sulphur, and sulphates in the soil, but the authors did not find a report of studies concerning activation of life in soils strongly acidified and polluted with sulphur. It is assumed that *Thiobacillus thiooxidans* was the organism responsible for sulphur oxidation in our experiments. This organism commonly appears in organic and mineral soils strongly acidified (pH 2–3). It prevails in sulphur mines where H_2SO_4 concentration reaches 10%, and pH is 0.6.

The lowest pH value found in our studies was 1.2. It should, however, be added that the above pH was measured with the ratio of soil to 1 n KCl solution as 1:5. Under field and pot conditions the concentration of hydrogen ions is accordingly higher, and pH lower.

In pot and field experiments, bacteria and actinomycetes, except bacteria-oxidizing sulphur and reducing sulphates, vanished completely in the soil of high acidity from the mining field. Also a small number of fungi survived. Analogous conditions were found with the addition of 0.1 and 0.5% S in slightly loamy sandy soil. After being sown, seeds of various plants did not germinate, or died just after germination (experiment 5, pH — 3.4). Thus these soils are free from microorganisms and quite toxic for higher plants.

Partial and a thorough neutralization of soil the more so, rapidly brought back to life bacteria and actinomycetes. The growth of fungi became also more intensive. Azotobacter was an exception, which was not found in deacidified soil even to pH — 7.3. Azotobacter is known to be very sensitive to the acid reaction of the medium, and it is possible that cells of this bacterium died out in sulphur-affected soil. Nitrifiers, however, showed a great activity in all deacidified soils. In these soils also, the growth of cultivated plants was very good. Sufficient liming could thus completely restore the biological activity of soils destroyed by sulphuric acid. In soils in which satisfactory plant growth was observed, a greater number and activity of microorganisms was found.

CONCLUSIONS

1. Soil pollution by elemental sulphur caused an intensive acidification of the medium and a very sharp decrease of biological activity. The microorganisms which survived were sulphur oxidizing and sulphate-reducing bacteria and also a small number of fungi.

2. Neutralization of sulphuric acid by liming fully restored biological activity of the soil.

3. A simultaneous addition of sulphur and lime to slightly loamy, sandy soil did not limit but increased the microbiological activity. This was found in higher wheat crop.

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AKTYWNOŚĆ BIOLOGICZNA GLEB SILNIE ZASIARCZONYCH

Badania przeprowadzono na glebach silnie zasiarczonych i zakwaszonych (do pH 1,4). Gleby poddano intensywnemu wapnowaniu. W doświadczeniach polowych i wazonowych oznaczano: ogólną liczebność bakterii i promieniowców, grzybów, azotobaktera, nitryfikatorów, aktywność proteolityczną drobnoustrojów, aktywność amonifikatorów oraz liczebność bakterii utleniających siarkę i redukujących siarczany.

Stwierdzono, że intensywne wapnowanie gleb zasiarczonych przywraca im aktywność biologiczną.

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